RESEARCH ARTICLE

Chrysin and its relation with gastric cancer

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ABSTRACT
Treatment of cancer has recently become a main topic for researchers. The rate of this disease is extremely high. Recently, about 14.1 million new cases of cancer occurred globally. It caused about 8.2 million deaths of all human deaths. Chrysin has been the subject of many studies due to its anticancer activity and has an overexpressing effect on eIF4E. The expression of eIF4E is regularly observed in different types of cancer, making eIF4E an attractive target for anticancer drugs. Our results indicated important molecular mechanisms involved in the chrysin anticancer activity. We hope this review help to develop ways of improving the effectiveness of chrysin in the treatment of gastric and other human cancers.

Key words: Cancer, Chrysin, eIF4E, anticancer drugs

Introduction

Gastric cancer, a leading cause of cancer-related mortality worldwide, is the fourth most common type of cancer and the second leading cause of cancer-associated deaths (about 737,419 deaths annually) worldwide (Zhang, 2013). Despite marked improvements in surgical, chemo, radio and other adjuvant therapies, the five-year survival rate of patients at the advanced stage remains less than 20-25% (Avdulov et al. 2004). Some Asian countries, including Korea, Japan, and China have the highest gastric cancer rate worldwide (Jemal et al., 2011). Recently, emerging evidence has revealed that different genetic changes are involved in the progression of gastric cancer. It is very important to investigate the exact molecular mechanism of gastric cancer development for improved anticancer therapeutics (Liang et al., 2013). Chrysin, the focus of the present review, is a flavone. Flavones contribute importantly to nitrogen fixation and chemical defenses (Nijveldt et al., 2001). Recently, chrysin has emerged as potential drug therapy for different cancers especially gastric, leukemia and cervical cancer (Zhang et al., 2004). Chrysin, existing in different things such as honey and many plant extracts, is mainly known for its antioxidant and anti-inflammatory effect. Some studies have indicated that chrysin inhibits cell growth of cancer by inducing apoptosis (Khoo et al., 2010). Apoptosis is a very critical regulatory mechanism for cell growth. Most cancer cells lose the normal regulation of apoptosis. Therefore, inducing apoptosis is widely accepted as one of the most important approaches to cancer therapy (Fesik, 2005). Translation initiation is

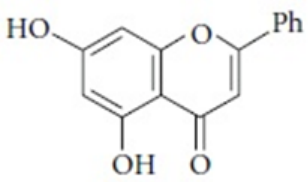

Figure 1. *The structure of chrysin (Menezes et al., 2016).*

deregulated in different human cancers such as gastric, breast, colorectal, head and neck, prostate, bladder, lung, cervical cancers and lymphomas (De Benedetti & Graff, 2004). Eukaryotic translation initiation factor 4E (eIF4E) is rate-limiting for cap-dependent translation. eIF4E plays an important role in several human tumors. The increase in eIF4E expression is commonly observed in several types of cancer, and therefore eIF4E could be an attractive anticancer drug target (Chen et al., 2004).

The objective of our study is to examine the expression of eIF4E in human gastric cancer cells. Also, we investigated the growth inhibitory effect of chrysin on gastric cancer cells. Our results identified the function of eIF4E and presented the eIF4E as a potential antitumor target in gastric cancer.

Composition of chrysin

Chrysin 5, 7 dihydroxyflavone at positions 5 and 7 of the A ring (Figure 1), is a natural flavonoid (Weng et al., 2005). Flavonoids are a family of natural phenolic compounds frequently found in vegetables, fruits, and especially honey. Flavonoids are generally safe and have low toxicity, and hence could be considered as cancer chemopreventive agents (Abolhasani et al., 2014). It has some activities such as anti-inflammatory and antioxidant also promotes apoptosis with disturbing cell cycle progression. Flavonoids can be simply absorbed, and a high level of flavonoids in food has been identified as a critical component of the human diet. More than 4,000 types of biologically active flavonoids have been identified (Nijveldt et al., 2001), which can be further divided into flavones, flavonols, flavanols, flavanones, isoflavonoid and anthocyanidins. Flavonoids are intriguing compounds because of their ability to act as cancer toxins and as cancer preventive agents (Ramos, 2008). Furthermore to their ability to prevent the progression of cancer, several naturally happening flavonoids have been shown to protect against the adverse effects of common chemotherapy agents (Psotová et al., 2004).

Chrysin with antioxidant and anti-inflammatory effects (Abolhasani et al., 2014) affects the apoptotic process on many types of cell lines (Sawicka et al., 2012). It has been recently shown as a potent inhibitor of aromatase (Ozkul et al., 2005) and human immunodeficiency virus activation in latent infection (Spiljac et al., 2016). Aromatase is a member of the cytochrome P450 superfamily that can irreversibly convert androstenedione and testosterone into estrone and estradiol (Séralini & Moslemi, 2001). The enzyme is expressed in many tissues in human, including hypothalamus, amygdala, and hippocampus (Brodie & Njar, 2000). These areas are crucial to neuroendrocrine regulation of reproduction and behavior (Conley & Hinshelwood, 2001). In vivo, chrysin has been demonstrated to inhibit tumor angiogenesis, which is a main step in metastasis (Fu et al., 2007). Chrysin may inhibit chymotrypsin-like and trypsin-like proteasomes, which contribute importantly to regulating apoptosis and the cell cycle (Wu & Fang, 2010). In vitro, chrysin suppresses the expression of HIF-1a in tumor cells (Fu et al., 2007). In a study by Bielak-Żmijewska, chrysin induced apoptosis in U937 cells through the inactivation of PI3K/Akt signal pathway as well as downregulation of NF-κB and IAP activation, and hence stimulated caspase3 which plays a crucial role in cell death (Bielak-Żmijewska, 2003). An increase is typically observed in cancer cells because of the activity of PI3K and Akt. As Bielak-Żmijewska indicated, U937 cells treated for 12 h with chrysin released cytochrome C from the mitochondria into the cytoplasm. Woo et al. concluded that chrysin, as a natural, nontoxic substance, is a potentially important agent to be used in the prevention of leukemia or therapy of patients with leukemia (Woo et al., 2004).

Chrysin contributed to the intrinsic pathway of apoptosis in HCT116, human colorectal cancer cells, human liver cancer cell line HepG2, and CNE-1, human nasopharyngeal carcinoma cells (Li et al., 2010). The percentage of apoptotic HCT116, HepG2 and CNE-1 cells increased markedly after curing by 1 ng/ml TNFα together with 10, 20 and 40 μM of chrysin. Chrysin crucially sensitises TNFα-induced apoptosis via a caspase cascade- activation of caspase3 and caspase8. Pretreatment of HCT116 cells with 40 μM chrysin and 1 ng/ml TNFα compared to the TNFα only inhibits IkB kinase activity, NF-κB transcriptional activity and suppresses anti-apoptotic gene c-FLIP. The above-cited study indicates that chrysin, related to TNFα in suppressive effect on NF-κB activation, decreases c-FLIP expression in HCT116 cells (Sawicka et al., 2012). This study increases our view of the molecular mechanism involved in chrysin anticancer activity.

Apoptotic effects of chrysin

Chrysin has been shown to inhibit proliferation and induced apoptosis in most studied cancer cells. Many studies of the mechanism of action suggest that chrysin is likely to act via caspase activation and inactivation of the Akt signaling. The mechanism of induction of apoptosis should be clarified further, although most studies have confirmed that chrysin induces apoptosis in different tumor cell lines (Menezes et al., 2016). Caspases are a conserved family of

enzymes that commit cells to programmed cell death. Caspase3 is known as an effector caspase among the 11 identified human caspases (Riedl & Shi, 2004). Proteolytic activation of caspase3 leads to the cleavage of poly (ADP-ribose) polymerase (PARP), a DNA repair protein as well as a protein to maintain genomic DNA integrity (Krishnakumar and Kraus 2010). An increase in cleaved PARP and cleaved caspase3 levels is indicative of cells undergoing apoptosis. Phan et al. recommended both ATC cell lines, in comparison with DMSO control group, revealed an increase in cleaved caspase3 accompanied when treated with chrysin with an increase in cleaved PARP in a dose-dependent method (Phan et al., 2011). ATC is a fast-growing malignancy, without differentiation and novel therapeutic strategies are needed. Recently, there have been many ongoing studies investigating the anticancer effects of chrysin because of its nontoxic properties as a natural flavonoid. In a study by Yin et al., the chrysin growth inhibitory effects on an anaplastic cell line of thyroid cancer (ARO81-1) were reported (Yin et al., 1999). However, with a recent DNA profiling analysis, ARO81-1 has been identified as a colon cancer cell line (Schweppe et al., 2008).

A study by Zhang et al. indicated that chrysin and its derivatives had critical anticancer effects on human cervical carcinoma (Zhang et al., 2004). Another study showed that chrysin potentially induced p38 and therefore activated NF-κB/p65 in the HeLa cells. The MAPK p38 pathway has been implicated in the regulation of a wide spectrum of cellular processes, including cell-cycle arrest and apoptosis. Besides, it has been regarded as a potential phosphate donor for the p65 subunit of NF-κB (Niedzwiecka et al., 2002). In a study of human leukemia cells, 22 different flavonoids were screened. Among the flavonoids tested, some of them were found to significantly decrease the cellular viability of the U937 cells. Chrysin was the most effective flavonoid to decrease the viability of the U937 cells with an IC_{50} of 16 μM. Chrysin also potentiated the effects in triggering apoptosis in the cells of TNFα (Woo et al., 2004). Also, Monasterio et al. reported that flavonoids, including chrysin, via a mechanism that required the activation of caspase3 and caspase8 induced apoptosis (Monasterio et al., 2004). In a study by Parajuli et al. chrysin exhibited tumor effects in a different range of human cell lines, including breast cancer (MDA-MB-231), prostate cancer cells (PC3) and malignant glioma cells (U87-MG and U-251). They extracted chrysin and other flavonoids from Scutellaria plants and showed dose-dependent inhibition of U87-MG proliferation (Bielak-Żmijewska, 2003). In a study in Iran on human prostate cancer by Samarghandian et al., chrysin induced apoptosis and cell cycle arrest in a prostate cancer cell line (Samarghandian et al., 2011). Li et al. by molecular mechanisms involved and assessing the sensitization effect of chrysin on TNFα-mediated apoptotic cell death, attempted to further address the anticancer role of chrysin (Li et al., 2010). Such sensitization which in turn leads to reduced expression of the anti-apoptotic NF-κB target gene, c-FLIP-L, one of the key anti-apoptotic genes capable of blocking caspase8 activity, is closely associated with its inhibitory effect on NF-κB activation (Parajuli et al., 2009). Other studies have shown that chrysin can potentiate the cytotoxicity of anticancer drugs by depleting cellular glutathione (GSH), an important factor in antioxidant defense. Chrysin potentially induces p38, activating NF-κB/p65 in HeLa cells (von Brandenstein et al., 2008).

Effect of chrysin in different types of Cancer

As we discussed in previous parts, chrysin has apoptotic effects on human cancer cells. Some of the effects and the molecular mechanisms involved are as follows:

In gastric cancer, 8-bromo-5-hydroxy-7-methoxychrysin and 5, 7-dimethoxy-8-iodochrysin have the strongest activities against HT-29 and SGC-7901 cells, respectively. The compound 5, 7-Dihydroxy-8-nitrochrysin has strong activities against both SGC-7901 and HT-29 cells (Woo et al., 2004).

In cervical cancer, chrysin inhibited proliferation and induced apoptosis in HeLa cells, though the effects were not as potent as those of its synthetic derivative compounds (Zhang et al., 2004). Chrysin (30 μM) potentially induced p38 and NF-κB/p65 activation in HeLa cells (von Brandenstein et al., 2008).

In leukemia, chrysin acts as the highest potent flavonoid to decrease cell viability and induced apoptotic in U937 cells (Woo et al., 2004). Chrysin induced apoptosis in Bcl-2 overexpressing U937 leukemia cells and was associated with activation of caspase3 and PLC-γ1 degradation. The induction of apoptosis was accompanied by downregulation of XIAP and inactivation of Akt (Monasterio et al., 2004). Chrysin, alone or in combination with other compounds, decreased Akt phosphorylation and potentially caused mitochondrial dysfunction in THP-1 and HL-60 leukemia cells (Ramos & Aller, 2008). Also, chrysin had the ability to abolish SCF/c-Kit signaling by inhibiting the PI3K pathway in MO7e, myeloid leukemia cells (Lee et al., 2007).

In breast, carcinoma and prostate cancer, chrysin showed dose-dependent inhibition of U87-MG, MDA-MB-231, U-251 and PC3 proliferation, and displayed apoptotic activity in U87-MG cells. However, the study did not report details about the apoptotic activity of chrysin in U-251, MDA-MB-231 and PC3 cells (Parajuli et al., 2009).

In colon cancer, chrysin caused the SW480 cells in cell cycle arrest at the G2/M phase in a dose-dependent manner (Wang et al., 2004).

In lung cancer, chrysin treatment increased extracellular GSH levels 11.2-fold, 5.1-fold, 3.0-fold and 1.5-fold in A549, H157, H1975 and H460 cells, respectively, as compared to untreated controls after 8 hours. By the 72-hour time point, extracellular GSH levels maintained an increase of approximately 9.7-fold, 5.0-fold, 3.9-fold and 2.4-fold in A549, H157, H1975 and H460 cells, respectively (Brechbuhl et al., 2012).

In thyroid cancer, chrysin inhibited proliferation of HTH7 and KAT18 in a dose and time-dependent manner. HTH7 and KAT18 cells with chrysin treatment had an important increase in cleaved caspase3, cleaved PARP, along with a decrease in cyclin D1, Mcl-1 and XIAP (Phan et al., 2011).

eIF4E

Aberrations in the control of mRNA translation initiation have been documented in many human cancers including gastric, breast, head and neck, colorectal, bladder, prostate, lung, cervical cancers and lymphomas (De Benedetti & Graff, 2004). Control of mRNA translation contributes critically to cell growth, proliferation and differentiation. In eukaryotes, most mRNAs are translated in a cap-dependent manner. The cap structure m^7GpppN is found at the 50 termini of all cellular eukaryotic mRNAs (Gingras et al., 1999). mRNA 5' cap-binding protein eIF4E plays a crucial role in the regulation of translation and is the rate-limiting member of the eIF4F complex (Mamane et al., 2004). eIF4F is composed of eIF4G, eIF4A, and eIF4E (von Brandenstein et al., 2008). eIF4A, a 46kDa RNA helicase, is an ATP-dependent helicase, and a large scaffolding protein; eIF4G, a 185kDa protein that co-localizes all of the other proteins involved in mRNA recruitment on the 40S subunit (Marcotrigiano et al., 1999), acts as a docking site for other proteins. The cellular levels of eIF4E molecules are 10 to 30-fold lower than other known initiation factors (Hiremath et al., 1985) and therefore its association with the eIF4F complex is the rate-limiting step in translation initiation (De Benedetti & Graff, 2004); however, the stoichiometry of eIF4F components is still debated by some investigators (Rau et al., 1996). It was previously hypothesized that an increase in the rate of translation has an impact on the spectrum of mRNAs synthesized (Lodish, 1974). eIF4E was initially assumed to be a single protein, partially because only a single 25 kDa polypeptide was obtained from mammalian sources by affinity chromatography. Ravel laboratory discovered that wheat germ contained two versions of eIF4E (Browning et al., 1987), eIF4E and eIF(iso)4E. It has been reported that *Arabidopsis thaliana* expresses not only eIF4E and eIF(iso)4E but also nCBP (Ruud et al., 1998); *Homo sapiens* expresses a second family member, 4EHP (Rom et al., 1998); and *Caenorhabditis elegans* expresses three eIF4E family members, IFE-1, 2, and 3 (Jankowska-Anyszka et al., 1998). It is now recognized that the wheat germ "oddity" is the norm; virtually all eukaryotes express multiple eIF4E family members. Multiple family members have been found for other initiation factors as well, e.g. eIF4A and eIF4G (Rhoads, 2009). eIF4E and Caping process dissociated and requires an internal RNA structure termed internal ribosome entry site (IRES) to which the 40S subunit binds directly. Originally, this mode of translation initiation was identified in pico RNA viruses, but subsequent studies revealed the presence of IRES-dependent cellular translation in mitosis and apoptosis (Jang et al., 1988).

An unusual feature of the eIF4E sequence is the high content of Trp residues. Some of these are involved in the binding of the eIF4G and cap (Marcotrigiano et al., 1999). These Trp residues allowed Joshi et al to discern a core region with consensus sequence H(X_5) W(X_2) W($X_{8–12}$) W (X_9) F(X_5) FW(X_{20}) F(X_7) W(X_{10}) W($X_{9–12}$) W($X_{34–35}$) W($X_{32–34}$) H in seven well established eIF4Es. They used this to subdivide eIF4E family members into three classes according to the residues corresponding to Trp-43 and Trp-56 of *Homo sapiens* eIF4E-1. Class I members at both positions contain Trp; Class II members, such as 4EHP, nCBP, IFE-4, and d4EHP, contain Tyr, Phe, or Leu at the first position and Tyr or Phe at the second position; and Class III members at the first position contain Trp and Cys or Tyr in the second position (Joshi et al., 2005).

It has been proposed that the translational efficiency of mRNA with highly complex 5′ untranslated regions (UTRs) is especially dependent on eIF4E levels. It was shown that overexpression of eIF4E could increase the 5′ UTR (Koromilas et al., 1992) such as chloramphenicol acetyltransferase and ornithine decarboxylase (Rousseau *et al.* 1996). Early studies demonstrated that eIF4E overexpression resulted in the transformation of immortalized cell lines as exemplified by increased proliferation, anchorage-independent growth, and invasiveness (Lazaris-Karatzas et al., 1990). The overexpression of eIF4E has been found recently in primary human malignancies such as the colon (Rosenwald et al., 1999), non-Hodgkin lymphoma (Wang et al., 1999), breast (Kerekatte et al., 1995), chronic myelogenous leukemia and acute myelogenous leukemia (Topisirovic et al., 2003). These data prompted different laboratories to investigate the role of eIF4E in neoplastic transformation as well as suggested the feasibility of targeting this molecule as a novel therapeutic approach. The impact of overexpressing eIF4E has been examined by several investigators in transgenic mouse models and can increase the incidence of multiple malignancies including lymphomas (Wendel et al., 2004). eIF4E overexpression leads to selective translation of mRNA such as cyclin D1, Bcl-2, Bcl-xL and VEGF in vitro (Li et al., 2003). The eIF4E

expression shows a significant correlation with cyclin D1 and VEGF expression in human tumors (Yang et al., 2007). eIF4E enhances nucleocytoplasmic transport for selected mRNA such as cyclin D1. Thus, eIF4E expression affects the expression of important regulators of cell growth and survival. In vitro, eIF4E overexpression mediates growth, proliferative and survival signaling, and has transforming activity in fibroblasts and mammary epithelial cells (De Benedetti & Graff, 2004). Transgenic eIF4E expressing mice showed a marked increase in tumorigenesis and developing tumors of various histologies. Thus, eIF4E also directly acts as an oncogene in vivo (Ruggero et al., 2004). In other studies, Waskiewicz et al. and Fukunaga and Hunter indicated that eIF4E was phosphorylated by the MNK1/2 serine/threonine kinases, which are activated in response to mitogenic and stress signaling downstream of ERK1/2 and p38 MAP kinase, respectively (Fukunaga & Hunter, 1997; Waskiewicz et al., 1997). eIF4E phosphorylation at serine 209 by MNK1/2 promotes its transformation activity (Wendel et al., 2007).

The disruption of translation initiation using RNA interference through the modulation of either eIF4E protein levels or formation of the eIF4F ternary complex has been examined (De Benedetti et al., 1991). Graff et al. demonstrated that intravenous administration of antisense oligonucleotides could successfully silence the expression of eIF4E in vivo in a mouse xenograft model. This decrease in eIF4E levels led to an inhibition of tumor growth and reduced cell viability. While the decrease in eIF4E levels was not restricted to only the tumor cells, the clinical impact on normal tissues was minimal as there were no significant changes in body weight, liver weight, or hepatic enzymes. These data demonstrated that modulating eIF4E protein levels was a useful approach to disrupting tumor growth in vivo without significant off-target effects in normal tissues. The function of eIF4E may also be interrupted by small molecule inhibitors that mimic the 5 methyl-7-GTP moiety found within the capped structure of mRNA. The small molecule ribavirin has been shown to suppress eIF4E-induced transformation through a mechanism in which the inhibitor competes with the endogenous 5 methyl-7-GTP cap structure for eIF4E. Through this competitive inhibition, Kentsis et al. were able to demonstrate a decrease in the translation of oncogenic messages such as ornithine decarboxylase as shown by the absence of its mRNA in the polysomal fraction of ribosomes (Kentsis et al., 2004).

Role of eIF4E in Translation

The mechanism through which only a subset of mRNAs is selectively translated upon overexpression of eIF4E could not be immediately explained. The most straightforward mechanism is that features in the UTRs specify which transcripts are selected to be translationally activated as eIF4E increases (Koromilas et al., 1992). In a study by Hamilton et al. Among transcripts which were translationally activated, fifty-nine terminal oligopyrimidine (TOP) and mospolyadenylation response element were enriched. The TOP sequence is present at the 59 ends of all mammalian ribosomal protein mRNAs and several translation factor mRNAs and plays a critical role in their translational regulation (Hamilton et al., 2006). Interestingly, only a subset of TOP sequences could confer differential eIF4E responsiveness (Mamane et al., 2007).

Effects of eIF4E in apoptosis

Apoptosis plays an essential role in the development and maintenance of homeostasis and protection against viral infection (Wyllie, 1993). Apoptosis is characterized by distinct morphological features, including chromatic condensation, cell and nuclear shrinkage, membrane blebbing, and oligonucleosomal DNA fragmentation (Wyllie, 1997). eIF4E prevents apoptosis in cells. The identification of novel anti-apoptotic eIF4E targets such as BI-1 (Chae et al., 2004), dad1 and surviving (Altieri, 2003) could explain the anti-apoptotic activity of eIF4E. It was relevant to show induction of eIF4E protects cells from apoptosis. Free eIF4E levels are commonly elevated in cancers due to an increase in PI3K/AKT/mTOR signaling or overexpression of eIF4E (Avdulov et al., 2004). Increased eIF4E expression contributes to tumor formation and progression in leukemias and lymphomas and several cancers (De Benedetti & Graff, 2004). In tumors, increased eIF4E function enhances the translation of select mRNAs (Mamane et al., 2007). Cellular mRNAs could be categorized into two groups; strong mRNA with relatively short, unstructured 5´ UTRs, and weak mRNA with lengthy, highly structured 5´ UTRs (Koromilas et al., 1992). The crucial difference between strong and weak mRNAs relates to weak mRNAs' much more sensitivity to eIF4E. Weak mRNAs usually encode growth and survival factors whose levels of expression are good indicators of eIF4E-relevent experimental cancer models (Graff et al., 2008). When activated, eIF4E disproportionally and dramatically stimulates translation of a limited and defined set of mRNAs encoding cancer-related proteins that control cell proliferation and viability. Increase in eIF4E function can enable the nucleocytoplasmic transport of potent growth regulatory proteins selectively (Culjkovic et al., 2006). Also, this function enhances the ribosome loading of mRNAs with lengthy GC-rich 5′UTRs, many of which encode potent growth and survival factors involved in malignancy. Interestingly, the majority of mRNAs, characterized by short, unstructured 5′UTRs, are mainly unchanged by changes in eIF4E activity (De Benedetti & Graff, 2004). eIF4E overexpression also stops the activation of pro-caspase12 and

pro-caspase3. When 3T3- tTA and 3T3-tTA-eIF4E cells were induced for 16 h and then treated with ionomycin, pro-caspase12 and pro-caspase3 cleavages to cause their active forms were not observed in induced 3T3- tTA-eIF4E cells. By comparison, pro-caspase cleavage was easily recognized in induced 3T3-tTA and uninduced 3T3-tTA-eIF4E cells (Mamane et al., 2007). As a result of proteins involved in the malignant tumor, eIF4E changing principally cause the protein expression of survival and cell growth potent regulators (Mamane et al., 2004).

Conclusion

Our review suggests that chrysin has antiproliferative effects on gastric cancer cells although the previous studies suggest chrysin, as a potent inhibitor of aromatase, may inhibit tumor cell progression, may be useful as a potential chemotherapeutic anticancer drug and may be a potential compound for cancer prevention and treatment. Further investigation, especially in vivo, is needed to support the use of chrysin in cancer. On the other hand, eIF4E plays a pivotal role in tumor formation and metastasis, and mainly in apoptosis. Since eIF4E has overexpression in several human cancers, its downregulation and downstream make it a prime target for anticancer therapies.

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